18 PANCURONIUM AND ROCURONIUM ANALYSIS BY LCMS

18.1 Summary

18.1.1 Quaternary nitrogen muscle relaxants (pancuronium and rocuronium) are extracted from biological samples using acetonitrile precipitation, solid phase extraction (SPE) and analyzed by high performance liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS). Pancuronium, rocuronium and the internal standard, verapamil, are extracted and analyzed simultaneously. LC-ESI-MS analysis is achieved with a 10-90% acetonitrile gradient containing 0.1% trifluoroacetic acid.

18.2 Specimen Requirements

18.2.1 2 mL blood, biological fluid or tissue homogenate.

18.3 Reagents and Standards

18.3.1 Ammonium carbonate

18.3.2 Ammonium acetate

18.3.3 Methanol

18.3.4 Acetonitrile

18.3.5 Hexane

18.3.6 Pancuronium bromide (Pavulon®, Baxter, 1 mg/mL)

18.3.7 Pancuronium bromide (Sigma)

18.3.8 Rocuronium bromide (Zemuron®, Organon, 10 mg/mL)

18.3.9 Verapamil hydrochloride (e.g. Alltech)

18.3.10 Trifluoroacetic acid

18.3.11 Glacial Acetic Acid

18.3.12 Potassium hydroxide

18.4 Solutions, Internal Standards, Calibrators and Controls

18.4.1 1.0 M Acetic Acid: Pipet 57.5 mL glacial acetic acid into a 1L volumetric flask. QS to volume with dH_2O.

18.4.2 Ammonium Acetate Buffer (pH 5.0, 50mM): Weigh 3.85 g ammonium acetate. Transfer to 1 L volumetric flask and add approximately 900 mL dH_2O. Adjust pH to 5.0 with 1.0 M acetic acid. QS to volume with dH_2O.

18.4.3 5.0 M Potassium hydroxide : Weigh 28 g potassium hydroxide. Transfer to 100 mL volumetric flask and QS to volume with dH_2O.

18.4.4 Ammonium Carbonate Buffer (pH 9.3, 0.01M): Weigh 0.47 g ammonium carbonate. Transfer to 500 mL volumetric flask and add approximately 450 mL dH_2O. Adjust pH to 9.3 with 5 M potassium hydroxide. QS to volume with dH_2O.

18.4.5 0.1M acetic acid in methanol: Pipet 10 mL 1.0 M acetic acid into a 100 mL volumetric flask. QS to volume with methanol. Prepare fresh daily.

18.4.6 Working standard solutions for pancuronium and rocuronium

18.4.6.1 100 µg/mL pancuronium/rocuronium working solution: Pipet 1 mL of 1 mg/mL stock solution of pancuronium and 100 µL of 10 mg/mL stock solution of rocuronium into a 10 mL volumetric flask. QS to volume with acetonitrile. Prepare fresh daily.

18.4.6.2 10 µg/mL pancuronium/rocuronium working solution: Pipet 1 ml of 0.1 mg/mL pancuronium/rocuronium working solution into a 10 mL volumetric flask. QS to volume with acetonitrile. Prepare fresh daily.

18.4.7 Quality Control (QC) solution

18.4.7.1 100 µg/mL pancuronium/rocuronium QC solution: Pipet 100 µL of 1 mg/mL stock solution of pancuronium and 10 µL of 10 mg/mL stock solution of rocuronium (both from different manufacturer or lot number than standards). Add 890 µL acetonitrile. Prepare fresh daily.

18.4.8 Internal standard working solution

18.4.8.1 10 µg/mL verapamil: Pipet 100 µL of 1 mg/mL verapamil stock solution into 10 mL volumetric flask and QS to volume with acetonitrile. Store in freezer.

18.4.9 Calibrators. To prepare the following calibration curve, pipet the following volumes into appropriately labeled 16 x 125 mm screw cap tubes

18.4.9.1 Cal 1: 10 mg/L pancuronium/rocuronium: 20 µL each of 1 mg/mL pancuronium and rocuronium
18.4.9.2 Cal 2: 5 mg/L pancuronium/rocuronium: 10 µL each of 1 mg/mL pancuronium and rocuronium
18.4.9.3 Cal 3: 2 mg/L pancuronium/rocuronium: 400 µL of 0.01 mg/mL pancuronium/rocuronium solution
18.4.9.4 Cal 4: 1 mg/L pancuronium/rocuronium: 200 µL of 0.01 mg/mL pancuronium/rocuronium solution
18.4.9.5 Cal 5: 0.5mg/L pancuronium/rocuronium: 100 µL of 0.01 mg/mL pancuronium/rocuronium solution
18.4.9.6 Cal 6: 0.1mg/L pancuronium/rocuronium: 20 µL of 0.01 mg/mL pancuronium/rocuronium solution

18.4.9.7 Add 2 mL blank blood to each tube.

18.4.10 Pancuronium and rocuronium control (QC)

18.4.10.1 1mg/L pancuronium/rocuronium QC: Pipet 200 µL of 0.01 mg/mL pancuronium/rocuronium QC solution into appropriately labeled 16 x 125 mm screw cap tube and add 2 mL blank blood.

18.4.10.2 Negative control: blood bank blood (or equivalent) previously determined not to contain rocuronium, pancuronium or verapamil.

18.5 Apparatus

18.5.1 Test tubes, 16 x 125 mm round bottom, screw cap with Teflon caps

18.5.2 Test tubes, 16 x 114 mm glass centrifuge, conical bottom

18.5.3 Centrifuge capable of 2000-3000 rpm

18.5.4 Nitrogen evaporator with heating block

18.5.5 Vortex mixer

18.5.6 GC autosampler vials with inserts

18.5.7 Solid Phase Extraction manifold

18.5.8 Strata C18-E SPE columns (6 ml, bed volume 500 mg), Phenomenex

18.5.9 LC/MS: Agilent Model 1100 LC-MSD

18.5.10 LCMS Instrument Conditions. The following instrument conditions may be modified to adjust or improve separation and sensitivity.

18.5.10.1 Elution conditions:

18.5.10.1.1 Column: Agilent Hypersil BDS 125 mm X 3 mm, 3 µm particle size

18.5.10.1.2 Column thermostat: 35° C

18.5.10.1.3 Solvent A: Water with 0.1%Trifluoroacetic acid

18.5.10.1.4 Solvent B: Acetonitrile

18.5.10.1.5 Gradient elution, stop time: 13.00 min

Time	Solv. B	Flow
0.00	10.0	0.65
8.00	90.0	0.65
9.00	10	0.65

18.5.10.2 Spray Chamber

- Ionization Mode: Electrospray
- Gas Temperature: 350° C
- Drying Gas (N_2): 12.0 L/min
- Nebulizer pressure: 35 psig
- Vcap (Positive): 4000 V

18.5.11 Selected Ion Monitoring

18.5.11.1 Polarity: Positive

18.5.11.2 SIM parameters (quantitation ions)

Rocuronium ions: 358, 413, 487, 529
Pancuronium ions: 412, 472, 571, 685
Verapamil IS ions: 165, 303, 455

18.6 Procedure

18.6.1 Label 16 x 125 mm screw cap tubes appropriately (blank, calibrators, controls and case sample IDs).

18.6.2 Prepare calibrators and controls.

18.6.3 Pipet 2 mL of each case specimen into appropriately labeled tubes.

18.6.4 Add 100 µL 0.01 mg/mL verapamil working internal standard solution to each tube. Vortex briefly.

18.6.5 Slowly, add dropwise 2 mL cold (freezer temperature) acetonitrile to each tube while vortexing. Continous vortexing, not mere mixing, is essential.

18.6.6 Vortex an additional 30 seconds.

18.6.7 Cap tubes.

18.6.8 Place tubes in freezer for at least 30 minutes to facilitate separation.

18.6.9 Centrifuge at approximately 2500 rpm for 15 minutes.

18.6.10 Transfer top (acetonitrile) layer to clean 16x125 mL tubes taking care not to transfer any lower layers.

18.6.11 Add 4 mL 0.01 M ammonium carbonate buffer to each tube. Vortex briefly.

18.6.12 Prepare C18-E SPE columns

18.6.12.1 Add 2 mL methanol to each column. Aspirate slowly under vacuum (approx 1 mL/min).

18.6.12.2 Add 4 mL ammonium carbonate buffer to each column. Aspirate slowly under vacuum.

18.6.13 Load buffered sample supernatants to columns. Aspirate slowly under vacuum.

18.6.14 Wash columns with 4 mL ammonium carbonate. Aspirate slowly under vacuum.

18.6.15 Repeat wash with 4 mL ammonium carbonate. Aspirate slowly under vacuum.

18.6.16 Add 500 µL hexane to each column. Aspirate. Dry the columns at > 10 inches of Hg for at least 10 minutes.

18.6.17 Elute drugs by adding 4 mL of freshly prepared 0.01M acetic acid in methanol. Collect eluants under gravity (no vacuum) into conical bottom screw cap tubes.

18.6.18 Evaporate eluants to dryness at approximately 50° C under nitrogen.

18.6.19 Reconstitute samples in 1 mL acetonitrile. Vortex briefly to ensure recovery of drugs from glass tube.

18.6.20 Evaporate samples again to dryness at approximately 50° C under nitrogen.

18.6.21 Reconstitute samples in 100 µL acetonitrile. Vortex briefly. Transfer to GC microvials.

18.6.22 Inject 5 µL of each sample on LC/MS in the API-ES/SIM Mode

18.7 Calculation

18.7.1 Drug concentrations are calculated by linear regression analysis using the ChemStation software.

18.8 Quality Control and Reporting

18.8.1 See Toxicology Quality Guidelines

18.9 References

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